Supporting information

How to design selective ligands for highly conserved binding sites: A case study using *N*-myristoyltransferases as model system

Christian Kersten,[ab] Edmond Fleischer,[a] Josef Kehrein,[bc] Christoph Borek,[a] Elmar Jaenicke,[d] Christoph Sotriffer,[c] Ruth Brenk[b,*]

[a] Institute of Pharmacy and Biochemistry, Johannes Gutenberg-Universität Mainz, Staudingerweg 5, 55128, Mainz, Germany

[b] Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5020, Bergen, Norway.

[c] Institute of Pharmacy and Food Chemistry, Julius-Maximilians-Universität Würzburg, Am Hubland, 97074, Würzburg, Germany

[d] Institute of Molecular Biophysics, Johannes Gutenberg University, Jakob-Welder-Weg 26, 55128, Mainz, Germany.

[*] corresponding author: ruth.brenk@uib.no

Table S 1: Thermodynamic characterization of compounds **1**-**5** binding to *Lm*NMT and *Hs*NMT1. Values are mean with standard error of at least duplicate determination.

Compound	*Lm*NMT				*Hs*NMT1			
	K_d [nM]	ΔG° [kJ/mol]	ΔH° [kJ/mol]	-TΔS° [kJ/mol]	K_d [nM]	ΔG° [kJ/mol]	ΔH° [kJ/mol]	-TΔS° [kJ/mol]
1	132 ± 23	-38.7 ± 0.4	-34.2 ± 0.7	-4.5 ± 1.1	248 ± 49	-37.2 ± 0.4	-19.2 ± 2.1	-18.0 ± 2.1
2	6.8 ± 2.7	-46.8 ± 1.3	-33.1 ± 0.8	-13.7 ± 2.0	5.6 ± 1.1	-46.5 ± 0.5	-25.5 ± 0.2	-20.9 ± 0.5
3	88.5 ± 39.0	-40.6 ± 1.8	-55.6 ± 2.3	15.0 ± 4.1	20.7 ± 1.9	-43.2 ± 0.2	-34.8 ± 0.8	-8.3 ± 1.1
4	21.4 ± 2.8	-44.2 ± 0.5	-44.3 ± 1.0	0.1 ± 1.5	184 ± 23	-37.8 ± 0.3	-16.9 ± 0.7	-21.0 ± 1.0
5	780 ± 509	-34.8 ± 2.1	-26.3 ± 2.4	-8.5 ± 4.5	$88{,}5\times10^3$ ± $40{,}8\times10^3$	-23.3 ± 1.1	-9.2 ± 3.4	-14.0 ± 2.3

Table S 2: Data collection and refinement statistics. Numbers in parenthesis characterize the highest resolution shell.

Complex	***Hs*NMT1-2**	***Hs*NMT1-4**	***Hs*NMT1-6**
PDB entry	6FZ3	6FZ5	6FZ2
Data collection			
Wavelength [Å]	0.976251	0.976251	1.54179
Space group	C 1 2 1	C 1 2 1	C 1 2 1
Unit cell parameters			
a, b, c [Å]	91.38, 58.21, 152.74	92.39, 58.16, 154.00	92.53, 58.29, 153.90
α,β,γ [°]	90.00, 92.28, 90.00	90.00, 92.36, 90.00	90.00, 92.52, 90.0
Resolution [Å]	76.60-2.00 (2.05-2.39)	76.94-1.89 (1.93-1.89)	16.80-2.05 (2.10-2.05)
No. unique reflections	46006 (3522)	62186 (4069)	51372 (2596)
R_{merge}	0.100 (0.388)	0.085 (0.551)	0.139 (0.680)
I/σI	4.5 (2.1)	6.4 (1.7)	6.2 (1.9)
Completeness [%]	84.9 (89.8)	94.8 (96.9)	99.3 (99.6)
Redundancy	1.9 (1.9)	2.6 (2.6)	3.6 (3.6)
Refinement			
Resolution [Å]	76.60-2.00	76.94-1.89	16.80-2.05
R_{work}/R_{free} [%]	0.200 / 0.237	0.189 / 0.223	0.237 / 0.278
No. atoms / average B-factor [$Å^2$]			
Protein	6102 / 29.34	6128 / 34.02	5976 / 34.06
Ligands	206 / 29.33	218 / 40.71	194 / 34.17
Water molecules	181 / 24.05	385 / 34.57	256 / 31.09
R.m.s deviations			
Bonds [Å]	0.0100	0.0093	0.0099
Angles [°]	1.476	1.440	1.446
Ramachandran plot			
favoured regions [%]	97.8	98.0	96.8
allowed regions [%]	2.0	2.0	2.4
outlier regions [%]	0.3	0	0.8

Table S 2 (cont.)

Complex	***Hs*NMT1-8x**	***Lm*NMT-4**	***Lm*NMT-6**
PDB entry	6F56	6EU5	6EWF
Data collection			
Wavelength [Å]	0.966000	0.966000	0.966000
Space group	P 21 21 21	P 1 21 1	P 1 21 1
Unit cell parameters			
a, b, c [Å]	58.19, 159.13, 174.99	48.77, 90.59, 53.42	46.39,90.22, 52.53
α,β,γ [°]	90.00, 90.00, 90.00	90.00, 114.14, 90.00	90.00, 111.91, 90.00
Resolution [Å]	87.50-1.94 (1.97-1.94)	48.75-1.50 (1.52-1.50)	48.74-1.54 (1.56-1.54)
No. unique reflections	112254 (4257)	65618 (3245)	59058 (2944)
R_{merge}	0.100 (0.839)	0.036 (0.526)	0.034 (0.479)
I/σI	11.3 (2.1)	16.8 (2.1)	15.7 (2.2)
Completeness [%]	92.8 (72.2)	96.3 (95.1)	98.8 (99.3)
Redundancy	6.5 (5.7)	3.1 (2.8)	3.0 (3.0)
Refinement			
Resolution [Å]	87.50-1.94	48.75-1.50	48.74-1.54
R_{work}/R_{free} [%]	0.200 / 0.239	0.169 / 0.205	0.170 / 0.194
No. atoms / average B-factor [$Å^2$]			
Protein	12590 / 34.40	3383 / 24.2	3375 / 21.2
Ligands	310 / 32.90	96 / 23.4	90 / 21.6
Water molecules	464 / 36.5	227 / 31.7	214 / 29.4
R.m.s deviations			
Bonds [Å]	0.0030	0.0068	0.016
Angles [°]	0.6900	0.9100	1.440
Ramachandran plot			
favoured regions [%]	97.2	97.8	97.6
allowed regions [%]	2.6	2.2	2.4
outlier regions [%]	0.2	0.0	0.0

Table S 3: Michaelis-Menten enzyme kinetic constants (K_M) for *Lm*NMT, *Hs*NMT1 and their variants. Results are mean values with standard deviation of at least two experiments. *Hs*NMT1 6x contains the 6 mutations W297F:A452M:L453V:L462V:L495M:Q496L and *Hs*NMT1 8x contains the 8 mutations R295Q:W297F:A452M:L453V:L462V:N473H:L495M:Q496L. n. d. = not determined due to enzyme inactivity.

NMT	K_M [µM]
*Lm*NMT	5.8 ± 3.4
*Lm*NMT H398N	3.1 ± 1.8
*Lm*NMT M420L	n.d.
*Lm*NMT L421Q	4.4 ± 2.1
*Lm*NMT H398N:M420L:L421Q	n.d.
*Hs*NMT1	2.6 ± 0.9
*Hs*NMT1 L495M	1.6 ± 0.3
*Hs*NMT1 Q496L	2.9 ± 1.4
*Hs*NMT1 N473H:L495M:Q496L	2.6 ± 0.8
*Hs*NMT1 R295Q	2.9 ± 0.4
*Hs*NMT1 R295Q:N473H:L495M:Q496L	3.9 ± 1.9
*Hs*NMT1 A452M	2.2 ± 0.3
*Hs*NMT1 L453V	2.1 ± 0.8
*Hs*NMT1 A452M:L453V	7.0 ± 1.5
*Hs*NMT1 A452M:L453V:L462V	3.6 ± 2.1
*Hs*NMT1 A452M:L453V:L495M	4.4 ± 2.0
*Hs*NMT1 6x	3.9 ± 0.9
*Hs*NMT1 8x	1.5 ± 0.4

Table S 4: Thermodynamic profile of compounds **1** and **4** binding to *Hs*NMT1 active site mutants. Values are mean with standard error of triplicate determinations.

	***Hs*NMT1 N473H:L495M:Q496L**				***Hs*NMT1L495M**			
Com-pound	**K_d [nM]**	**ΔG° [kJ/mol]**	**ΔH° [kJ/mol]**	**-TΔS° [kJ/mol]**	**K_d [nM]**	**ΔG° [kJ/mol]**	**ΔH° [kJ/mol]**	**-TΔS° [kJ/mol]**
1	234 ± 8	-37.2 ± 0.1	-22.8 ± 0.1	-14.4 ± 0.1	605 ± 265	-35.5 ± 1.3	-19.4 ± 1.0	-16.2 ± 2.3
4	13.6 ± 4.1	-44.5 ± 1.0	-18.1 ± 0.2	-26.3 ± 0.8	78.1 ± 32.6	-40.3 ± 0.9	-15.5 ± 0.9	-24.8 ± 1.2

Table S 5: Backbone 1D-RMSD of 50 ns MD production runs of NMTs in complex with MyrCoA and with or without inhibitors. Values are mean with standard deviation compared to energy-minimized starting structures. [a]Structure generated from docking pose. [b]Structure generated from direct transfer of known crystal structure (PDB code of template binding mode in parenthesis).

NMT complex	Starting structure (PDB code)	RMSD
*Lm*NMT	3H5Z[32]	1.04 ± 0.12
*Lm*NMT	4CGP[36]	1.24 ± 0.14
*Lm*NMT-**1**	2WSA	1.01 ± 0.13
*Lm*NMT-**2**	2WSA[a]	1.00 ± 0.10
*Lm*NMT-**3**	2WSA[a]	1.03 ± 0.10
*Lm*NMT-**4**	2WSA[a]	1.18 ± 0.14
*Lm*NMT-**5**	4CGN[36]	0.97 ± 0.10
*Lm*NMT-**6**	5A27[37]	1.15 ± 0.13
*Lm*NMT-**6a**	5A27	1.05 ± 0.09
*Hs*NMT1	3IU1, chain B	1.42 ± 0.14
*Hs*NMT1	4C2Y, chain A[45]	1.24 ± 0.14
*Hs*NMT1-**1**	3IWE, chain A	1.04 ± 0.09
*Hs*NMT1-**2**	3IWE, chain A[a]	1.08 ± 0.11
*Hs*NMT1-**3**	3IWE, chain A[a]	1.07 ± 0.09
*Hs*NMT1-**4**	3IWE, chain A[a]	1.29 ± 0.12
*Hs*NMT1-**5**	3IU1, chain B[a]	1.32 ± 0.16
*Hs*NMT1-**5**	4C2Y, chain A [b] (4CGN)	1.15 ± 0.13
*Hs*NMT1-**6**	4C2Y, chain A[a] (5A27)	1.22 ± 0.13
*Hs*NMT1-**6a**	4C2Y, chain A[a] (5A27)	1.85 ± 0.20
*Hs*NMT1-8x	3IU1, chain B	1.41 ± 0.15
*Hs*NMT1 L495M	3IU1, chain B	1.42 ± 0.13
*Hs*NMT1 L495M-**1**	3IWE, chain A	1.60 ± 0.11
*Hs*NMT1 L495M-**4**	3IWE, chain A (*Hs*NMT1-**4**)	1.15 ± 0.12

Table S 6: Side-chain order parameters (S^2) of C-terminus and preceding residue derived from MD simulations.

S^2	*Lm*NMT		*Hs*NMT1		*Hs*NMT1 L495M	
Ligand	M420	L421	L495	Q496	M495	Q496
none	0.17	0.86	0.78	0.34	0.48	0.58
none	0.32	0.87	0.63	0.16	-	-
1	0.28	0.92	0.53	0.45	0.17	0.20
2	0.27	0.92	0.75	0.11	-	-
3	0.19	0.90	0.76	0.18	-	-
4	0.34	0.92	0.57	0.75	0.32	0.63
5	0.43	0.93	0.68	0.75	-	-

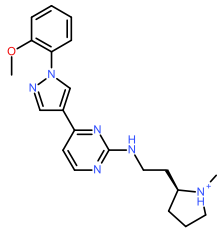
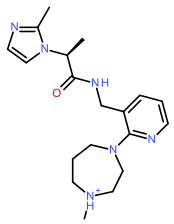
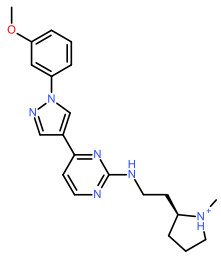
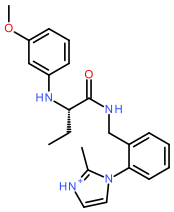
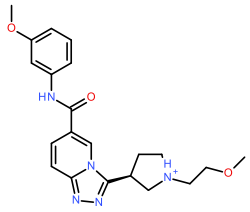
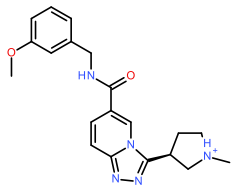
Table S 7: Sidechain order parameters S^2 of binding-site residues derived from MD simulations. For Gly, Ala and Trp no S^2 are available.

				*Lm*NMT							*Hs*NMT1							*Hs*NMT1 L495M		
***Lm*NMT**		***Hs*NMT1**		**apo**	**apo**	**1**	**2**	**3**	**4**	**5**	**apo**	**apo**	**1**	**2**	**3**	**4**	**5**	**apo**	**1**	**4**
TYR	80	TYR	180	0,89	0,87	0,92	0,92	0,92	0,91	0,91	0,85	0,88	0,91	0,91	0,90	0,90	0,90	0,87	0,91	0,90
VAL	81	VAL	181	0,91	0,90	0,93	0,93	0,89	0,75	0,92	0,80	0,81	0,91	0,92	0,76	0,90	0,83	0,19	0,91	0,91
GLU	82	GLU	182	0,36	0,54	0,48	0,32	0,19	0,19	0,29	0,48	0,24	0,74	0,35	0,39	0,37	0,20	0,27	0,34	0,47
ASP	83	ASP	183	0,82	0,77	0,84	0,85	0,24	0,53	0,83	0,51	0,32	0,83	0,58	0,69	0,42	0,76	0,18	0,52	0,42
ASP	84	ASP	184	0,71	0,34	0,63	0,74	0,72	0,82	0,60	0,46	0,52	0,67	0,73	0,73	0,79	0,66	0,28	0,76	0,71
PHE	88	PHE	188	0,22	0,30	0,38	0,21	0,62	0,67	0,35	0,54	0,21	0,16	0,08	0,33	0,36	0,72	0,30	0,13	0,33
ARG	89	ARG	189	0,87	0,41	0,89	0,44	0,48	0,89	0,81	0,37	0,54	0,64	0,67	0,33	0,69	0,36	0,35	0,46	0,51
PHE	90	PHE	190	0,10	0,06	0,25	0,14	0,19	0,36	0,36	0,31	0,37	0,33	0,35	0,25	0,85	0,32	0,30	0,46	0,82
TYR	92	TYR	192	0,77	0,74	0,89	0,88	0,61	0,89	0,91	0,79	0,85	0,86	0,81	0,83	0,83	0,84	0,77	0,83	0,83
PHE	96	PHE	196	0,78	0,82	0,90	0,91	0,83	0,91	0,89	0,80	0,85	0,83	0,82	0,83	0,84	0,81	0,79	0,79	0,82
ILE	166	ILE	245	0,77	0,77	0,82	0,73	0,53	0,78	0,57	0,86	0,86	0,88	0,88	0,83	0,83	0,87	0,87	0,83	0,86
ASN	167	ASN	246	0,82	0,82	0,88	0,88	0,86	0,88	0,88	0,47	0,81	0,85	0,82	0,80	0,84	0,84	0,12	0,85	0,83
PHE	168	PHE	247	0,84	0,82	0,88	0,86	0,87	0,85	0,88	0,59	0,84	0,88	0,86	0,87	0,42	0,88	0,30	0,87	0,51
TYR	202	TYR	281	0,87	0,88	0,87	0,85	0,85	0,86	0,90	0,83	0,84	0,85	0,85	0,85	0,86	0,85	0,86	0,86	0,84
THR	203	THR	282	0,34	0,90	0,93	0,92	0,92	0,91	0,91	0,55	0,32	0,89	0,89	0,83	0,81	0,84	0,87	0,90	0,66
ALA	204	ALA	283																	
GLY	205	GLY	284																	
VAL	206	VAL	285	0,31	0,15	0,91	0,91	0,91	0,91	0,90	0,65	0,87	0,90	0,89	0,89	0,45	0,89	0,19	0,90	0,37
TYR	217	TYR	296	0,68	0,75	0,82	0,83	0,88	0,90	0,89	0,32	0,81	0,88	0,83	0,74	0,87	0,79	0,78	0,77	0,90
PHE	218	TRP	297	0,84	0,77	0,85	0,70	0,79	0,88	0,69										
HIS	219	HIS	298	0,62	0,63	0,82	0,85	0,83	0,79	0,47	0,29	0,31	0,83	0,83	0,67	0,85	0,83	0,50	0,84	0,85
PHE	232	PHE	311	0,22	0,54	0,71	0,21	0,58	0,42	0,28	0,68	0,34	0,23	0,26	0,32	0,38	0,26	0,25	0,65	0,64
TYR	326	TYR	401	0,87	0,87	0,88	0,90	0,88	0,88	0,89	0,89	0,88	0,88	0,89	0,89	0,85	0,90	0,86	0,89	0,86
ILE	328	LEU	403	0,24	0,27	0,91	0,91	0,70	0,91	0,91	0,38	0,66	0,87	0,87	0,83	0,89	0,87	0,25	0,85	0,68
SER	330	SER	405	0,31	0,19	0,69	0,75	0,24	0,63	0,64	0,31	0,77	0,49	0,70	0,26	0,81	0,24	0,39	0,56	0,84
THR	331	THR	406	0,76	0,55	0,72	0,23	0,80	0,84	0,72	0,32	0,23	0,40	0,61	0,33	0,60	0,15	0,36	0,35	0,43
LEU	341	LEU	416	0,71	0,68	0,75	0,73	0,76	0,77	0,76	0,19	0,32	0,52	0,63	0,53	0,63	0,39	0,17	0,64	0,57
ALA	343	ALA	418																	
TYR	345	TYR	420	0,87	0,84	0,92	0,92	0,89	0,92	0,92	0,86	0,87	0,90	0,91	0,88	0,90	0,89	0,84	0,90	0,91
VAL	346	SER	421	0,93	0,93	0,93	0,94	0,93	0,92	0,94	0,88	0,87	0,86	0,87	0,86	0,87	0,89	0,88	0,87	0,88
VAL	374	VAL	449	0,92	0,69	0,93	0,93	0,93	0,93	0,93	0,81	0,91	0,89	0,80	0,85	0,92	0,91	0,89	0,90	0,91
ASN	376	ASN	451	0,74	0,61	0,79	0,83	0,61	0,78	0,76	0,63	0,70	0,72	0,77	0,69	0,80	0,72	0,64	0,77	0,82
MET	377	ALA	452	0,69	0,77	0,93	0,92	0,58	0,90	0,93										
VAL	378	LEU	453	0,27	0,15	0,38	0,15	0,43	0,50	0,44	0,85	0,87	0,73	0,73	0,81	0,66	0,89	0,84	0,79	0,85
GLY	395	GLY	470																	
ASP	396	ASP	471	0,43	0,39	0,60	0,46	0,71	0,48	0,54	0,47	0,48	0,65	0,58	0,66	0,76	0,46	0,37	0,58	0,74
GLY	397	GLY	472																	
HIS	398	ASN	473	0,29	0,30	0,31	0,43	0,48	0,54	0,38	0,29	0,14	0,24	0,17	0,22	0,52	0,30	0,12	0,44	0,43
LEU	399	LEU	474	0,59	0,84	0,93	0,92	0,85	0,92	0,92	0,61	0,83	0,92	0,91	0,65	0,78	0,76	0,83	0,91	0,57
ARG	400	GLN	475	0,83	0,84	0,83	0,80	0,79	0,81	0,83	0,39	0,59	0,54	0,62	0,40	0,60	0,72	0,43	0,65	0,48
TYR	401	TYR	476	0,48	0,85	0,86	0,87	0,88	0,91	0,88	0,29	0,61	0,89	0,89	0,86	0,90	0,88	0,89	0,90	0,88
VAL	419	VAL	494	0,87	0,88	0,88	0,90	0,89	0,89	0,91	0,88	0,86	0,70	0,80	0,88	0,89	0,84	0,88	0,87	0,59
MET	420	LEU	495	0,17	0,32	0,28	0,27	0,19	0,34	0,43	0,78	0,63	0,53	0,75	0,76	0,57	0,68	0,48	0,17	0,32
LEU	421	GLN	496	0,86	0,87	0,92	0,92	0,90	0,92	0,93	0,34	0,16	0,45	0,11	0,18	0,75	0,75	0,58	0,20	0,63

Table S 8: Calculated thermodynamic profiles of W1 derived from MD simulations using the *SPAM* approach.[43] Values are in kcal/mol. A W1 hydration site was considered at a density higher than 0.07 throughout the trajectory except for *Lm*NMT in complex with **3** for which a density cut-off of 0.05 was used. Starting structures are the same as described in Table S5.

NMT + Ligand	**ΔG_{SPAM}**	**ΔH_{SPAM}**	**$-T\Delta S_{SPAM}$**
*Lm*NMT apo	no W1 density peak found		
*Lm*NMT apo	no W1 density peak found		
*Lm*NMT + **1**	3.59	-3.32	6.91
*Lm*NMT + **2**	5.64	0.45	5.19
*Lm*NMT + **3**	4.26	-1.76	6.02
*Lm*NMT + **4**	5.67	-2.30	7.97
*Lm*NMT + **5**	no W1 density peak found		
*Lm*NMT + **6**	no W1 density peak found		
*Lm*NMT + **6a**	3.06	-0.65	3.70
*Hs*NMT1 apo	2.60	-2.69	5.29
*Hs*NMT1 apo	2.98	-3.16	6.14
*Hs*NMT1 + **1**	2.93	-3.93	6.76
*Hs*NMT1 + **2**	0.05	-5.02	5.07
*Hs*NMT1 + **3**	1.72	-2.24	3.96
*Hs*NMT1 + **4**	0.66	-3.11	3.78
*Hs*NMT1 + **5**	2.42	-4.98	7.40
*Hs*NMT1 + **5**	2.39	-4.59	6.98
*Hs*NMT1 + **6**	2.27	-4.39	6.66
*Hs*NMT1 + **6a**	2.92	-3.71	6.63

Table S 9: Structures and scores of tested compounds from virtual screening. Structures represent the docked stereoisomers, for testing only racemic mixtures were available.

Compound	Structure	ZINC ID	FlexX Score (Rank)	HYDE Score (Rank)
9		55060934	-34.9 (21)	-50 (3)
10*		67446715	-32.1 (63)	-49 (4)
11		20869258	-29.9 (99)	-45 (8)
12		55369988	-34.2 (28)	-53 (1)
13		96267504	-30.2 (91)	-44 (12)
14		96267435	-32.4 (49)	-43 (15)

* The compound that was docked contained a 2-methylimidazoyl group whereas in-house NMR analysis revealed that the purchased compound contained a 4-methylimidazoyl group.

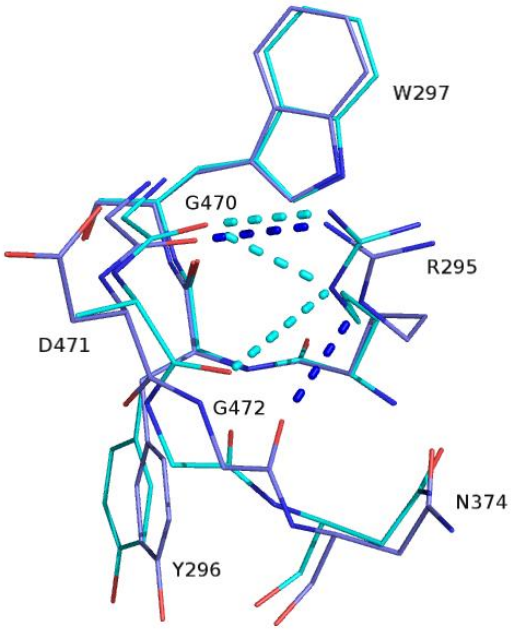


Figure S 1: Interactions of Arg295 with backbone oxygen atoms of Gly470, Asp471 and Gly472 in the *Hs*NMT1 open conformation (blue carbon atoms and hydrogen bonds indicated as dashes, PDB code 3IU1) and closed conformation (cyan carbon atoms and hydrogen bonds indicated as dashes, PDB code 3IWE).

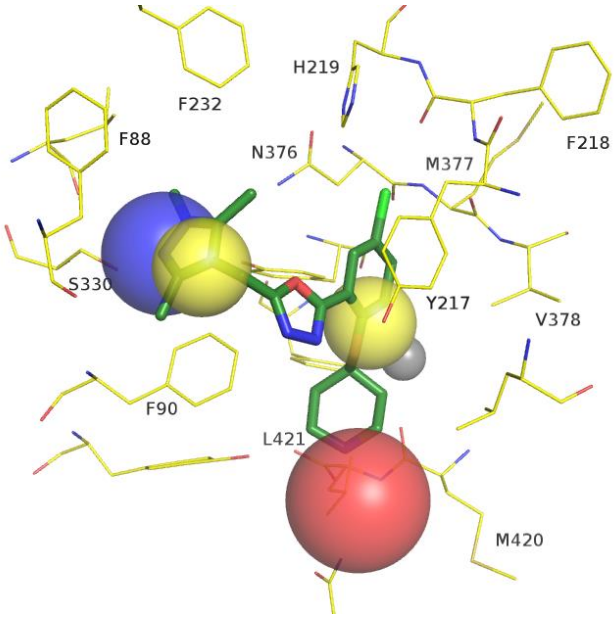


Figure S 2: Pharmacophore query used for virtual screening. For clarity, *Lm*NMT in complex with compound **7** (PDB code 5A28) is also shown. Blue sphere (radius 1.4 Å): hydrogen-bond acceptor; red sphere (radius 1.7 Å): basic atom/positive charge; grey sphere (radius 0.5 Å): any atom (selectivity marker); yellow spheres (radius 1 Å): aromatic atoms.

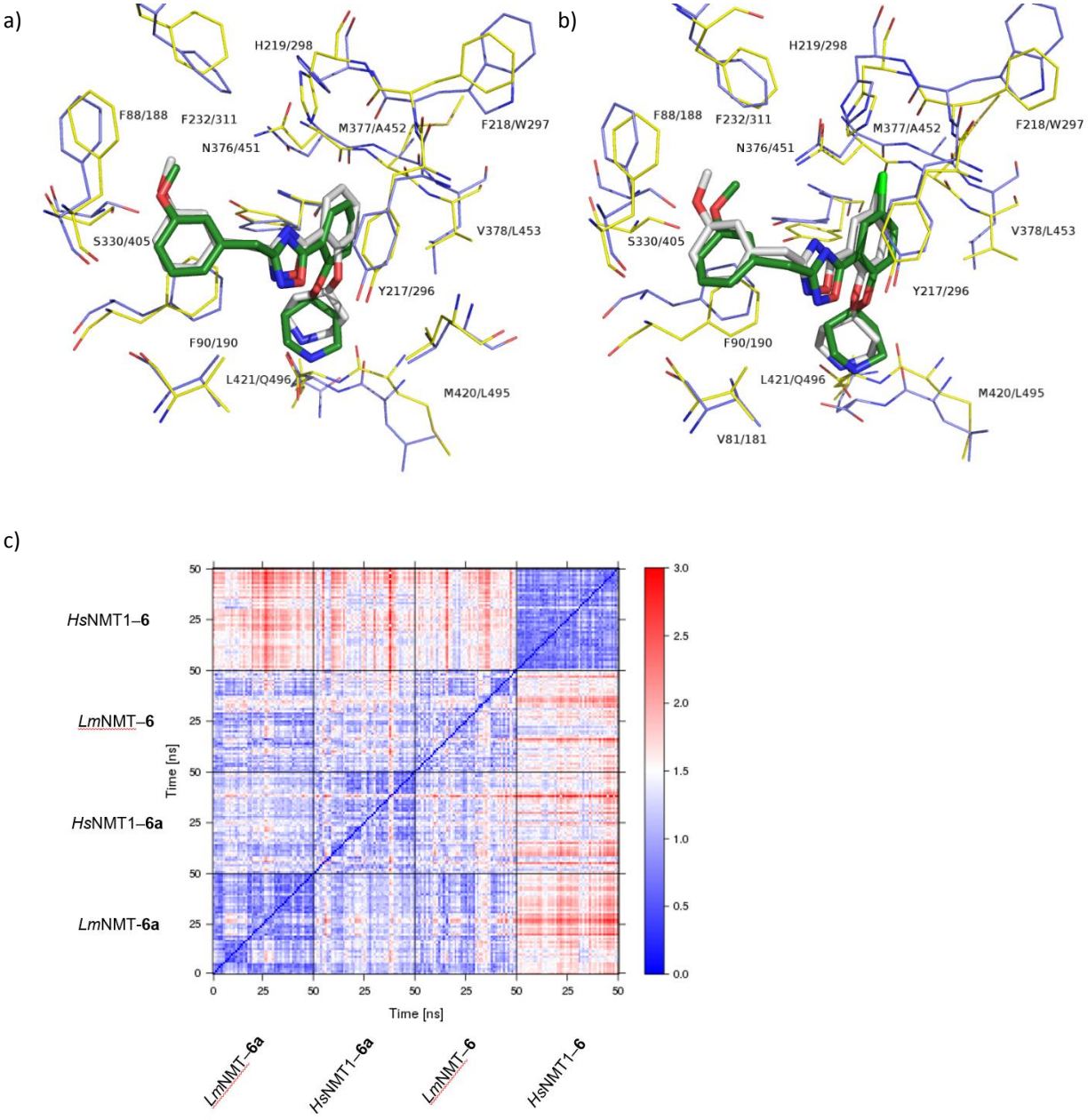


Figure S 3: Superposition of representative MD frames of *Lm*NMT (yellow carbon atoms and green ligand, derived from PDB code 5A27) and *Hs*NMT1 (light blue carbon atoms and white ligand, docking pose into PDB code 4C2Y) in complex with compound **6** (a) and **6a** (b) Residues are labeled as *Lm*NMT/*Hs*NMT1. c) 2D-RMSD plot of ligand **6** and **6a** heavy atoms (without chlorine) bound to *Hs*NMT1 and *Lm*NMT, respectively.